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Bone Morphogenetic Proteins in the Treatment of Non-unions and Bone Defects: Historical Perspective and Current Knowledge

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Abstract: Bone morphogenetic proteins (BMPs) are a family of bone matrix polypeptides which have been isolated from a variety of mammalian species, including man. BMPs initiate chondroblastic differentiation in pluripotent mesenchymal progenitor cells, followed by the synthesis of new bone by enchondral ossification. BMPs have demonstrated the ability to induce healing of osteoperiosteal defects in several animal models, and now in human studies, supporting a role in the reconstruction of bone defects. BMPs are responsible for the osteoinductive capacity of demineralized bone matrix (DBM) implants, which have also been demonstrated to be helpful in healing defects. Recent reports on the use of both purified, naturally occurring, and recombinant human bone morphogenetic proteins in the treatment of non-unions and bone defects have shown promising results. The use of bone morphogenetic protein implants to augment or replace autogenous and allogenous bone grafts will reduce morbidity and circumvent the risk of disease transmission associated with bone transplantation.

Segmental bone loss and non-union, whether after reconstructive surgery, lesion excision, or fracture, can present complex problems. An important part of the therapeutic approach to bone defects is the implantation of materials that support new bone formation. Such implants may hasten healing by three mechanisms: osteoconduction, osteogenesis, and osteoinduction.

In osteoconduction, implanted material serves as an inert scaffold for the ingrowth of host bone. This includes the differentiation and maturation within the implant of host osteoprogenitor cells, with ingrowth of vascular elements. Ideally, "creeping substitution" then replaces the implant with new bone to form a functional skeletal element [35,51,52]. Osteogenesis is the synthesis of new bone by surviving pre-osteoblasts and osteoblasts within a bone autograft. These cells proliferate and mature into centers of new bone formation. Osteoinduction is the formation of new bone by the active recruitment of host pluripotent cells that differentiate into chondroblasts and osteoblasts [35,51–53]. The ideal artificial implant would be both osteoinductive and osteoconductive; it would cause new bone to form, then support its replacement of the bone defect.

It is now well accepted that osteoinduction is controlled, at least in part, by bone matrix proteins often collectively referred to as bone morphogenetic proteins (BMPs). These proteins are low-molecular weight polypeptides that have been isolated from the bones of a variety of mammalian species, including mouse, rat, bovine, monkey, and man [11,20,36–41,46,48,55]. They are also produced by clonal osteogenic sarcoma cell lines [46,48].

In recent years, bone morphogenetic proteins have been recognized as a potentially powerful clinical tool. Research efforts have been devoted to elucidating their properties and exploring ways in which they may be used to augment or replace bone grafts.

Bone Grafts

Traditionally, the treatment of bone loss and non-union has included various types of bone grafts. Fresh autograft is the benchmark against which the performance of other implants is judged. Autograft acts by all three mechanisms of bone healing: surviving surface osteocytes produce early new bone [4], bone morphogenetic proteins in the matrix are osteoinductive, and the three-dimensional structure of cancellous bone supports new blood vessel and bone ingrowth [18]. The use of cancellous and corticocancellous autograft has generally been successful [14,18,19,23,34] but requires an additional operative procedure to obtain the bone graft, with considerable potential morbidity. In one study, 25% of patients having iliac crest autografts reported significant pain at an average of five postoperative years [45]. Six to 20% of patients will complain of pain, hypersensitivity or buttocks anesthesia, and 3 to 9% will suffer major complications [7,12,45,60]. Use of autograft bone can also be hampered by insufficient volume of tissue, especially in children and patients in whom previous graft harvesting has been performed.

Allograft bone is often used as an alternative to autogenous bone graft. However, non-demineralized allografts demonstrate essentially no osteogenicity or osteoinductivity. During the process of revascularization of allografts, the host may become sensitized to graft-derived antigens, with the resulting lymphoplasmacytic infiltration causing occlusion of local blood vessels preventing revascularization of the graft. The ensuing necrosis of the graft allows the proliferation of inflammatory granulation tissue, weakening the

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cortical component of the graft, and interfering with new bone formation and incorporation [2,3,18,24]. Therefore, fractures repair poorly since revascularization is impeded by inflammatory tissue [18]. Freezing and freeze-drying (lyophilization) appear to attenuate these responses, but they also diminish the mechanical strength of the graft. In addition, enthusiasm for allograft bone has been tempered by concern about the transmission of infectious agents, including the human immunodeficiency virus (HIV) [6].

Demineralized Bone Matrix (DBM)

The discovery of osteoinductive bone matrix proteins arose from an appreciation of the osteoinductivity of demineralized bone matrix implants. Considerable evidence suggests that DBM may represent an alternative to standard bone grafts.

The use of DBM implants in the reconstitution of bone defects dates back to the work of Senn (1889), who used the decalcified residue of ox bone to treat chronic osteomyelitic defects [43]. One of the first clinical uses of demineralized bone in the modern era was reported in 1961 by Sharrard and Collins [44], who successfully used EDTA-decalcified allograft bone for spinal fusion in children. This work was supported by contemporaneous animal studies by Ray and Holloway (1957) [33], Burger et al. (1962) [5], and Hejna and Ray (1963) [22].

In 1965, Urist [49] reported a landmark study in which consistent osteoinduction by acid-decalcified bone was obtained in animals, with meticulous attention to the details of processing, such as time, temperature, and HCl concentration. With this, the stage was set for further animal studies, which almost universally support the use of DBM as an aid to bone healing.

A number of studies have demonstrated the clinical potential of DBM implants in the treatment of segmental long bone defects. In 1968, Urist reported the use of surface-decalcified or totally decalcified bone in 26 patients receiving joint or spinal arthrodeses, or having non-unions [50]. Healing was observed in about 75% of patients, with no implant-related complications. A more recent report [17] described over 300 craniofacial, periodontal and orthopaedic lesions treated with DBM, with healing generally occurring within 3 to 6 months.

Disadvantages associated with DBM include its radiolucency, lack of inherent rigidity and strength, and the need for meticulous care in its preparation. Also, the degree of osteoinductivity of DBM implants pales in comparison to that of purified or recombinant BMPs.

Purified Bone Morphogenetic Proteins

It is now known that the osteoinductivity of DBM implants is attributable to bone matrix proteins that are exposed to the milieu by demineralization. DBM and bone morphogenetic proteins induce new bone formation by an enchondral process, in contrast to an osteoconductive response in which no chondroblastic phase occurs [15,35,37,42,52]. In brief, bone morphogenetic proteins initiate chondroblastic differentiation in pluripotent mesenchymal progenitor cells. This is followed by the appearance of cells with an osteoblastic phenotype, and their elaboration of osteoid upon the cartilage framework, which is resorbed.

Urist's landmark 1965 report [49] described ectopic bone induction using acid-decalcified bone matrix transplants, and convincingly established the osteoinductivity of devitalized, decalcified bone. The importance of this work lies in its carefully controlled demonstration that new bone can be induced independent of the bone tissue milieu. While Urist's early hypothesis that "substances or degradation products of dead tissue stimulate . . . primitive connective tissue cells to differentiate into osteoblasts,"[82] stopped short of postulating a specific diffusible osteoinductor, the work stimulated the search for such a substance in bone matrix.

The solubilization and extraction of bone morphogenetic proteins were first realized in 1979 by Urist et al. [54]. The product showed more bone morphogenetic activity than DBM, and was named bone morphogenetic protein (BMP). This was followed, in 1981, by the report of Sampath and Reddi [40] confirming that the post-extraction bone matrix was not osteoinductive in an *in vivo* ectopic assay, but that its osteoinductivity could be totally restored by reconstituting the matrix with the crude extract.

Numerous bone-inducing proteins have been isolated from bone and characterized. These preparations, variously called bone morphogenetic proteins (BMPs), osteoinductive factors, or osteogenin, were found to predictably induce ectopic enchondral bone formation in animals [13,35,37,39,40,42]. These ectopic site assays were crucial in establishing the true osteoinductive nature of the extracts tested, isolated from the factors that are present at an orthotopic site.

It has long been thought that a carrier material was necessary for the successful *in vivo* use of BMPs. Collagen has emerged as the most promising material for the delivery of BMPs. While it seems obvious that collagen would be a good delivery system for osteoinductive substances, since the mineralization of hard tissues normally occurs on a matrix of fibrillar collagen [29], the exact function of collagen remains uncertain. It has been suggested that post-translational phosphorylation of collagen chains modifies chain chemistry, creating sites of mineral nucleation on the surface of collagen fibers [16]. While collagen alone is not osteoinductive, it appears to provide an excellent osteoconductive substrate for new bone formation. Since DBM is mostly bone collagen and non-collagenous proteins, a composite implant of DBM and bone morphogenetic proteins to form a "super DBM" may be seen as advantageous. However, with the advent of recombinant human bone morphogenetic proteins, the possibility exists to completely avoid the problems associated with allograft materials by using synthetic carriers or purified bone collagens.

Naturally occurring BMPs have been evaluated in orthotopic animal bone healing models. Nilsson et al. [31] demonstrated the success of bovine BMP in a canine ulnar non-

union model. In this study, the BMP preparation, implanted with gelatin, showed the ability to induce bone defect healing over a twelve-week period, independent of bone matrix. Heckman et al. [21] used a canine radius model in which 12-week-old established non-unions were treated with partially purified canine BMP on a polylactic acid (PLA) carrier. This study more closely simulated the clinical problem of non-union, whereas earlier studies modeled primary treatment of bone loss. The BMP preparation was effective in producing some bridging of the defects by trabecular bone at twelve weeks, but did not yield mechanically effective unions. Though acknowledging potential problems with their carrier, the authors attributed weak new bone formation in part to species-specificity, an idea that continues to be controversial. The homology of the BMPs among various mammalian species [41] and the finding that pure recombinant human BMPs can induce bone defect healing in a variety of animal species [8–10,28,47,56,59,61] suggest that the proteins themselves are probably not appreciably species-specific. Proteinaceous impurities, however, can incite immunogenic responses that decrease the effectiveness of BMP implants.

The powerful osteoinductive effect of naturally occurring BMPs has been tested in the clinical arena, as well. Preliminary studies using partially purified, naturally occurring human BMP (hBMP) in the management of non-unions have been reported in three papers by Johnson et al. [25–27]. Twelve patients, with femoral non-unions refractory to standard measures, were treated with various combinations of internal fixation and autogenous or allogenous bone grafting plus implants of hBMP on a carrier of either gelatin or polylactic acid/polyglycolic acid (PLA/PGA). Eleven of 12 subjects achieved union, at an average of 4.7 months [25]. Another group of six patients with established tibial non-unions after failure of internal or external fixation received autogenous bone grafting and internal or external fixation, augmented with implants of hBMP on a PLA/PGA carrier. Union was achieved in all subjects, at an average of 5.7 months [26]. A third study involved 25 patients with refractory non-unions of the femur, tibia, or humerus. All were treated with autolyzed, antigen-extracted bone plus hBMP. Union was achieved in 24 of the 25 subjects [27]. No major complications or adverse reactions were observed during any of these human trials. While no definitive conclusions can be based upon these reports, since bone autografts were used and the studies were uncontrolled, the safety of the implants in humans was demonstrated. The investigators were confident that hBMP had played an important role in healing these long-standing non-unions.

Recombinant Bone Morphogenetic Proteins

The foregoing studies were haunted by the possibility that, in a relatively crude extract of bone matrix proteins, certain co-factors may be present which are required for BMP's osteoinductivity. Absolutely pure BMP extracts are difficult, if not impossible, to obtain. This question was answered with the cloning and expression of recombinant human bone morphogenetic proteins (rhBMPs) [38,55,57,58]. With the purification of human BMPs in sufficient quantity and purity to provide amino acid sequence data, cDNAs were isolated, cloned and expressed in host cells. To date, seven potentially bone morphogenetic proteins have been generated in this fashion, and four have shown bone morphogenetic activity in animals: BMP-2 (BMP 2a), BMP-4 (BMP-2b), BMP-5, and BMP-7 (OP-1) [8–11,28,36,38,55,56,59,61]. Currently, there are two recombinantly-produced bone morphogenetic proteins nearing FDA approval in the US: recombinant human bone morphogenetic protein-2 (rhBMP-2) and recombinant human osteogenic protein-1 (rhOP-1). The latter is a trade name for rhBMP-7. Both have been reported to induce healing of bone defects in animal models, and are in various stages of human trials.

Recombinant human BMP-2

Recombinant human BMP-2 has been tested in multiple orthotopic animal models. Toriumi et al. [47] used a canine mandibular defect model to test the efficacy of rhBMP-2. Histomorphometric analysis at six months revealed that 68% of the volume of the rhBMP-2 implants was replaced by mineralized bone, compared to less than 4% of control implants.

Yasko et al. [59] used a rat femoral model to test two doses of rhBMP-2, and compared them to implantation of guanidine-extracted demineralized rat bone matrix only. Both doses of rhBMP-2 induced enchondral bone formation in osseous defects in a dose-related manner. Only the higher dose resulted in union, suggesting concentration-dependence of the biological effect of BMP.

Zegzula et al. [61] examined the effect of rhBMP-2, delivered in a porous PLA implant, on bone formation in a critical-sized defect in the radial diaphysis of rabbits. Defects treated with rhBMP-2 healed as readily as defects filled with autograft. Histomorphometric data indicated that the amount of bone formation in the defects treated rhBMP-2 was equivalent to the amount in autograft-treated sites.

Welch et al. [56] studied the effects of rhBMP-2 in an absorbable collagen sponge (ACS) on bone healing in a goat tibia fracture model. Bilateral closed tibial fractures were created in 16 skeletally mature goats, and reduced and stabilized using external fixation. In each animal, one tibia received the study device, and the contralateral fracture served as control. The device was implanted as a folded onlay or wrapped circumferentially around the fracture. The rhBMP-2/ACS produced a significant increase in torsional toughness, and trends of increased torsional strength and stiffness compared to controls. The device placed in a wrapped fashion around the fracture produced significantly tougher callus compared to the onlay method. The increased callus volume associated with rhBMP-2 treatment produced only moderate increases in strength and stiffness.

Kirker-Head et al. [28] created 2.5-cm mid-diaphyseal segmental defects in the femora of sheep and stabilized them with stainless steel plates. Implants combining rh-

BMP-2 and poly[D,L-(lactide-co-glycolide)](PLA/PGA) bioerodible polymer were added. Three of seven treated sites healed. In the animals that healed, new bone mineral content equaled that of the intact femur by week 16, with recanalization of the medullary cavity approaching completion at week 52. The authors were encouraged by the performance of this implant in this demanding model. We hypothesize that one of the reasons for the relatively low rate of success is the use of the PLA/PGA carrier. The literature suggests that collagen is a superior carrier material for BMPs.

Recombinant Human OP-1

Cook et al. used a rabbit ulnar segmental defect model[1] to evaluate the ability of rhOP-1 to restore a segmental osteoperiosteal defect [8]. Animals receiving rhOP-1 were compared to animals receiving implants of naturally occurring bovine bone morphogenetic protein (bOP) (with same collagen carrier) and to animals receiving implants of rabbit DBM. The rhOP-1 sites showed complete radiographic bony union across the defect within eight weeks, with mechanical strength approaching that of intact ulnae. In addition, the rhOP-1 sites were superior to the other experimental sites.

The same authors reported on an ulnar segmental defect model in dogs [9]. Histologically, rhOP-1-treated sites examined at 16 weeks had new cortices composed of lamellar and woven bone, with normal-appearing marrow elements in the reconstituted medullary canal. Healing occurred more rapidly than with autograft in a comparable model [30] and more completely than with bovine BMP in a model [21] in which the defect site was smaller. Again, the unions achieved reached a level of mechanical strength approaching that of intact bone.

It has been recognized that a mammal's capacity for bone repair and regeneration is roughly inversely proportional to its position on the phylogenetic tree [51]. Thus, a prerequisite for use of rhOP-1 in man is the demonstration of its effectiveness in non-human primates. Cook et al [10]. reported on the use of rhOP-1 in African green monkeys. Five of six rhOP-1-treated ulnae, and three of five tibiae exhibited radiographic bridging by new bone, first seen at four weeks and completed by six to eight weeks. Histologic evaluation of rhOP-1 sites revealed areas of woven and lamellar bone, and normal marrow elements. For healed rhOP-1-treated ulnae, the average torsional strength to failure was 95% of control at twelve weeks; and, for rhOP-1-treated tibiae, the average strength was 68%.

A multicenter, randomized clinical trial prospectively comparing rhOP-1 to autograft in the treatment of tibial non-unions has been completed and is under FDA review. Thirty patients with 31 tibial non-unions were randomized, with no implant-related complications. There were two failures in the rhOP-1 group and one in the autograft group, in this difficult group of multiply-operated patients. All have radiographic evidence of new bone formation at their non-union sites, and most have returned to normal activity levels.

Future Directions

The potential use of BMPs in the treatment of non-unions and bone defects is limited only by our imaginations. Basically, any indication for bone grafting is a potential indication for BMPs. Bone morphogenetic protein implants may provide an alternative to the use of bone grafts in the reconstruction of bone defects caused by trauma, neoplasia or infection. The use of bone morphogenetic proteins to augment or replace bone graft will reduce the amount of surgery needed to treat such conditions, and circumvent viral transmission associated with transplantation of bone products. Unpublished work from the author's institution suggests that BMPs can be effectively combined with bulk freeze-dried allograft segments.

While animal studies performed to date seem to indicate that bone morphogenetic protein implants will effectively induce new bone formation in man, important questions remain. In general, larger, more phylogenetically-advanced animals exhibit less exuberant responses to bone morphogenetic implants than rats and rabbits, for example. It is possible that human patients will demonstrate an unpredictably sluggish response to recombinant human BMPs, although this is not suspected on the basis of available human cases. The possibility of immunogenic reactions must also be considered. While pure, recombinant proteins are unlikely to elicit an immune response, proteinaceous impurities either in BMPs or in carrier materials are a potential source of immunogenicity. Finally, the use of BMP implants must not be considered a substitute for vascularity, adequate soft tissue coverage, or bony stability.

Bone morphogenetic protein research has seen remarkable progress over a relatively brief period, culminating in recent years with the development of recombinant human BMPs. The impressive new bone formation induced by BMPs may soon have a major impact upon musculoskeletal surgery.

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